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A chromene and prenylated benzoic acid from *Piper aduncum*

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Abstract

In addition to nerolidol, 2′,6′-dihydroxy-4′-methoxydihydrochalcone, methyl 2,2-dimethyl-8-(3′-methyl-2′-butenyl)-2*H*-1-chromene-6-carboxylate, methyl 2,2-dimethyl-2*H*-1-chromene-6-carboxylate and methyl 8-hydroxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylate, two new natural products were isolated from the leaves of *Piper aduncum*, 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid and 3-(3′,7′-dimethyl-2′,6′-octadienyl)-4-methoxybenzoic acid. The structures of the isolates were established based on analysis of spectroscopic data, including ES–MS. The DNA-damaging activity of the isolated compounds was also investigated against mutant strains of *Saccharomyces cerevisiae*. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Piper aduncum*; Piperaceae; Chromenes; Prenylated benzoic acid; Dihydrochalcone; Nerolidol; DNA-damaging activity

1. Introduction

Chemical studies carried out on Piperaceae species have revealed the occurrence of a variety of compounds including essential oils, pyrones, lignoids and unsaturated amides (Parmar et al., 1997; Alécio, Bolzani, Young, Kato, & Furlan, 1998). The species *Piper aduncum* L. is used as remedy for stomach aches and as an insect repellent (Asprey & Thornton, 1954). Previous investigations on *P. aduncum* have described phenylpropanoids, such as myristicin and dillapiol, benzoic acid derivatives, chromenes and flavonoids (Burke & Nair, 1986; Orjala, Eldelmeier, Wright, Rali, & Sticher, 1993a; 1993b; Moreira, Guimarães, & Kaplan, 1998). During the course of chemical studies on *P. aduncum* we isolated seven compounds, including a new chromene **1** and a new benzoic acid derivative **5**. In the search for potential anticancer agents employing a mechanism-based yeast bioassay for DNA-modifying agents (Gunatilaka, Samaranayake, Kingston, Hofmann, & Johnson, 1992; Gunatilaka, Kingston, & Johnson, 1994), it was found that the chromenes 2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**1**), methyl 2,2-dimethyl-8-(3′-methyl-2′-butenyl)-2*H*-1-chromene-6-carboxylate (**2**), methyl 2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**3**), methyl 8-hydroxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**4**) and the benzoic acid derivative, 3-(3′,7′-dimethyl-2′,6′-octadienyl)-4-methoxybenzoic acid (**5**), were active. Although chromene and benzoic acid derivatives are frequently isolated from *Piper* species and many of them exhibit diverse biological activity such as antimicrobial, molluscicidal (Orjala et al., 1993b), germination inhibition, fungicidal (Nair & Burke, 1990) and insecticidal activities (Bernard et al., 1995), there is no previous report describing damaging effect on DNA. In this paper we report the structure elucidation for two new isolated compounds **1** and **5** and the growth inhibitory effects of compounds **1–5** against strains of *Saccharomyces cerevisiae*.

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Table 1
^1H and ^{13}C NMR (200 and 50 MHz, respectively, $CDCl_3$, J in Hertz) data for compounds **1** and **5**

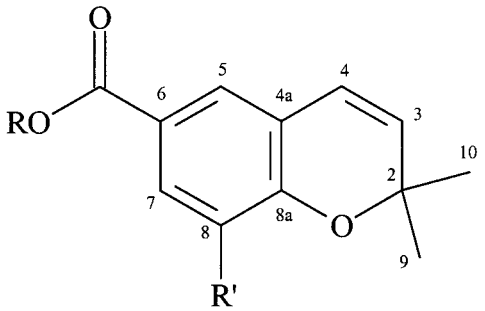
	1		**5**	
	H	C[a]	H	C[a]
1	–	–	–	122.5 (s)
2	–	77.2 (q)	7.88 d (2.2)	130.8[b] (d)
3	5.65 d (9.9)	131.0 (d)	–	129.7[b] (s)
4	6.34 d (9.9)	121.4 (d)	–	160.7 (s)
4a	–	120.7[c] (s)	–	–
5	7.72 d (2.0)	128.7 (d)	6.87 d (8.4)	109.2 (d)
6	–	121.5[c] (s)	7.96 dd (8.4, 2.2)	129.3 (d)
7	7.86 dd (8.4, 2.0)	131.8 (d)	–	–
8	6.79 d (8.4)	116.3 (d)	–	–
8a	–	157.9 (s)	–	–
9	1.46	28.4 (q)	–	
10	1.46	28.4 (q)	–	
1′	–	–	3.34 d (7.3)	28.0 (t)
2′	–	–	5.31 t (7.3)	121.6 (d)
3′	–	–	–	136.1 (s)
4′	–	–	2.07 m	39.8 (t)
5′	–	–	2.07 m	26.4 (t)
6′	–	–	5.10 t (7.2)	124.0 (d)
7′	–	–	–	131.0 (s)
8′	–	–	1.67 s	25.4 (q)
9′	–	–	1.71 s	15.8 (q)
10′	–	–	1.60 s	17.4 (q)
OCH_3	–	–	3.90 s	55.2 (q)
COOH	–	171.0 (s)	–	168.4 (s)

[a] Multiplicities for carbon signals were obtained by DEPT 135° experiments.
[b] Interchangeable assignments.
[c] Interchangeable assignments.

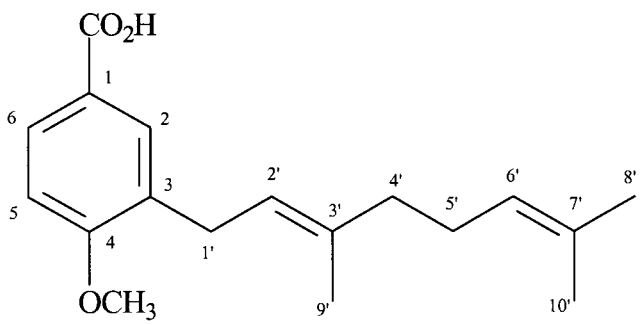
2. Results and discussion

A CH_2Cl_2-soluble part of MeOH extract of the leaves of *P. aduncum* was fractionated by CC on silica gel followed by HPLC to afford compounds **1**–**5**, nerolidol and 2′,6′-dihydroxy-4′-methoxydihydrochalcone.

2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**1**) was shown to have a molecular formula $C_{12}H_{12}O_3$ by analysis of its electrospray mass spectrum (ES–MS), ^1H and ^{13}C NMR spectra. Its IR spectrum revealed absorption bands at 2400–3400/1680 and 1600/1445 cm^{-1} characteristic of carboxylic acid and aromatic groups, respectively. Its ^1H NMR spectrum exhibited close similarities to those of 2,2-dimethyl-2*H*-1-chromene-6-carboxylate **3** (Burke & Nair, 1986) by the presence of two doublets at δ 5.65 and 6.34 (9.9 Hz) and a singlet at δ 1.46 (2 CH_3) and, as in the case of **3**, its spectrum showed three aromatic signals at δ 7.86 (dd, $J=2.0$ and 8.4 Hz), 7.72 (d, $J=2.0$ Hz) and 6.79 (d, $J=8.4$ Hz), corroborating the presence of a trisubstituted aromatic ring. As indicated by IR and by ^1H and ^{13}C NMR spectra (Table 1), the methyl ester function in **3** was replaced by a carboxylic acid function (δ_C 171.0) and the structure of compound **1** was determined as depicted.



	R	**R'**
1	H	H
2	CH_3	CH_2-CH=C(CH_3) (1′ 2′ 3′ 4′/5′)
3	CH_3	H
4	CH_3	OH



3-(3′,7′-dimethyl-2′,6′-octadienyl)-4-methoxybenzoic acid (**5**) has the molecular formula $C_{18}H_{22}O_4$ deduced by analysis of electrospray mass spectrum (ES–MS) and ^{13}C NMR data. In its IR spectrum, absorptions for an aromatic acid carbonyl group (1680 cm^{-1}) and an aromatic ring (1600, 1490 cm^{-1}) were present, while the UV spectrum displayed an absorption maximum at 254 nm (log ϵ 3.8) corresponding to the aromatic ring.

The ^1H NMR spectrum (Table 1) of **5** showed signals corresponding to three coupled aromatic resonances at δ 6.87 (d, 1H, $J=8.4$ Hz), 7.88 (d, 1H, $J=2.2$ Hz) and 7.96 (dd, 1H $J=8.4$, 2.2 Hz) for a 1,4,5-trisubstituted aromatic ring and δ_H 1.34 (1H, s); it also displayed a singlet at 3.90 (3H, s) for a carboxylic acid

function and an aryl methoxyl group, which were confirmed by the signals at δ_C 168.4 and 55.2, respectively, in its ^{13}C NMR spectrum. The monoterpene side chain signals were determined from DQCOSY and HETCOR spectra. Thus, the methylene protons H-1′ at δ 3.34 (2H, d, $J=7.3$ Hz) were coupled to the olefinic proton H-2′ at δ 5.31 (1H, t, $J=7.3$ Hz), which in turn showed an allylic coupling to the methyl group H-9′ at δ 1.71 (3H, s). The methine proton H-6′ at δ 5.10 (1H, t, $J=7.2$ Hz) was further coupled to the methylene proton H-5′ at δ 2.07 (2H, m). The *E* geometry for the $\Delta^{2'3'}$-double bond was established on the basis of the ^{13}C NMR data of the vinyl methyl group H-9′ (De Haan & van de Ven, 1973). The position of the monoterpene moiety was deduced by NOESY spectrum analysis and by comparison of the ^{1}H and ^{13}C NMR spectral data with those published for methyl 3-(3,7-dimethyl-2,6-octadienyl)-4-methoxybenzoate (Orjala et al., 1993b).

The known compounds 2,2-dimethyl-8-(3-methyl-2-butenyl)-2*H*-chromene-6-carboxylate (**2**), methyl 2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**3**), methyl 8-hydroxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**4**) and 2′,6′-dihydroxy-4′-methoxydihydrochalcone were previously isolated from the same source (Burke & Nair, 1986; Diaz, Arias, & Nathan, 1987; Orjala et al., 1993a; Moreira et al., 1998), while nerolidol (Ramos, Da Silva, Luz, Zoghbi, & Maia, 1986) was isolated for the first time from *P. aduncum*.

Compounds **1**–**5** were evaluated against genetically engineered mutants of yeast *Saccharomyces cerevisiae* and have showed IC_{12} values of 120 and 100; 130 and 100; 105 and 120; 110 and 120; 130 and 120 μg/ml against the mutant yeast strains RS 321N and RS 322YK (*rad* 52Y), respectively. Even though the activity could be considered weak, they were selective towards mutant strains lacking DNA cleavage repair mechanism since they were inactive ($IC_{12} > 1{,}000$ μg/ml) against the 'wild type' strain RS 188N (*rad* +) (Gunatilaka et al., 1992; 1994). As there is no previous report on the activity of chromene compounds on DNA, the data obtained here are the basis for our further search for antitumoral or fungicidal compounds in the Piperaceae.

3. Experimental

3.1. Instrumentation and chromatography materials

Silica gel (Merck 230–400 mesh) was used for all column chromatography unless otherwise stated and solvents were redistilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, using $CDCl_3$ as a solvent and TMS as reference. IR spectra were obtained on a Nicolet spectrometer. ES–MS were recorded on a VG Platform II spectrometer. HPLC separations were performed on a Shimadzu LC-10AS using a reversed phase column (Waters Nova Pak, C_{18}; 3.9×150 mm) eluted with MeOH:H_2O (3:2), flow rate of 0.5 ml/min and detection at 254 nm.

3.2. Plant material

Piper aduncum L. (Piperaceae) leaves were collected at Reserva da Ripasa, Ibaté, SP and identified by Dr. Guillermo E. D. Paredes (Universidad Pedro Ruiz Gallo, Lambayeque, Perú). The voucher specimen is deposited at Departamento de Ecologia, Instituto de Biociências-USP.

3.3. Bioassays

The screening methods utilizing mutant strains of *S. cerevisiae* have been described elsewhere (Gunatilaka et al., 1992). IC_{12} values refer to the concentration in μg/ml required to produce an inhibition zone of 12 mm diameter around a 100 μl well during a 48 h incubation period at 37°C. The positive control camptothecin was active at 5.0 μl/ml for RS 322YK and at 200 μl/ml for RS 188N; streptonigrin was active at 4.0 μl/ml for RS 321N.

3.4. Extraction and isolation of constituents

The CH_2Cl_2-soluble part of MeOH extract of powdered leaves of *P. aduncum* (24.0 g) was applied to a silica gel column (300 g), and eluted with hexane containing increasing volumes of EtOAc (up to 100%) to give 46 fractions (A_1–A_{46}). Fraction A_3 (961.5 mg) was applied to a silica gel column (2.5 g) 230–400 mesh, eluted with hexane containing increasing concentrations of EtOAc (up to 70%) to give 14 fractions (B_1–B_{14}). Fraction B_2 afforded compound **2** (13.0 mg). Fraction A_4 (218.0 mg) was chromatographed via silica gel CC, eluted with hexane containing increasing amounts of EtOAc (up to 70%) to give 10 fractions (C_1–C_{10}). Fraction C_5 (133.0 mg) yielded pure compound **3** (23.0 mg). Fraction A_5 was chromatographed on silica gel CC, eluted with hexane containing increasing amounts of EtOAc (up to 70%) to give nerolidol (180 mg). Fraction A_{11} (675.6 mg) yielded the pure compound **4** (171 mg). Fraction A_{13} (980.0 mg) was chromatographed on silica gel CC, eluted with hexane containing increasing amounts of EtOAc (up to 90%) to give the compound 2′,6′-dihydroxy-4′-methoxydihydrochalcone (408.0 mg). Fraction A_{14}, a green gum (25.0 mg), was further purified by reversed-phase HPLC, as described in the general experimental procedures, to give **5** (12.3 mg). The dried and powdered leaves (1.003 g) of *P. aduncum* were extracted

with EtOAc (five times, 150 ml each). The resulting extract (325.6 mg) was dissolved in $CHCl_3$ and partitioned with an aqueous solution of $NaHCO_3$ (six times, 50 ml each). The aqueous fraction was neutralized with HCl (6 N) and submitted to partition with EtOAc (three times, 100 ml each) to give a residue (248.0 mg). This fraction (248.0 mg) was submitted to a prep. TLC eluted with $CHCl_3$:MeOH (98:2) and HOAc (1%) to give 12.0 mg of compound **1**.

*2,2-Dimethyl-2*H*-1-chromene-6-carboxylic acid* (**1**): amorphous solid; UV (MeOH) λ_{max} ($\log \epsilon$): 240 nm (1.9); IR ν_{max} (KBr): 2400–3400, 3010, 2920, 2870, 1680, 1610, 1485, 1440, 1135 cm^{-1}; ES–MS *m/z* (rel int): 205 [M + 1] (100); ^1H and ^{13}C NMR, see Table 1.

3-(3′,7′-Dimethyl-2′,6′-octadienyl)-4-methoxybenzoic acid (**5**): amorphous solid; UV (MeOH) λ_{max} ($\log \epsilon$): 254 nm (3.8); IR ν_{max} (KBr): 2400–3400, 3005, 1680, 1640, 1600, 1488, 1445, 1130 cm^{-1}; ES–MS *m/z* (rel int): 303 [M + 1] (100); ^1H and ^{13}C NMR, see Table 1.

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